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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 43/00, 31/195

A1

(11) International Publication Number: WO 91/16075

(43) International Publication Date: 31 October 1991 (31.10.91)

(21) International Application Number: PCT/AU91/00155

(22) International Filing Date: 19 April 1991 (19.04.91)

(30) Priority data:
PJ 9726 20 April 1990 (20.04.90) AU

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(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.

(54) Title: BONE MARROW TREATMENTS

(57) Abstract

Haemotological malignancy in an animal is treated by using a polyvalent particle-emitting radionuclide to label a bone-localising chelating agent and administering this agent to affect bone marrow of the animal, but in a dosage close to but less than a level which will cause complete bone marrow ablation, and administering a cytotoxic pharmaceutical in a dose sufficient to affect bone marrow of the animal, but also in a dose close to but less than a level which will cause complete bone marrow ablation. Examples include the use of samarium-153 as the radionuclide and a radio pharmaceutical selected from EDTMP, DTPMP, HEEDTMP, NTMP, TTHMP, HEDP and physiologically acceptable salts thereof. The cytotoxic drug can be melphalan or a derivative or analogue thereof.

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BONE MARROW TREATMENTS

The present invention relates to bone marrow treatments, and more particularly, is concerned with a treatment in which ablation of bone marrow is achieved followed by bone marrow transplantation.

Such treatment is possible and can be a cure for many patients with haematological malignancy such as acute leukaemia and multiple myeloma. It has been found necessary to kill all bone marrow cells. This would be fatal to the patient but for subsequent bone marrow transplantation with healthy bone marrow. If less than all the bone marrow cells are ablated, then natural recovery mechanisms operate through cell regeneration and recurrence of malignancy is likely. Thus, the patient will only enjoy a period of remission. One established technique is to achieve bone marrow ablation with total body irradiation (T.B.I.). Bone marrow transplantation with healthy bone marrow can then take place almost immediately.

It has also been proposed to use chemoradiotherapy techniques to eradicate the haematological malignancy and this treatment immunosuppresses the patient to prevent rejection of the transplanted marrow. However, a significant proportion of patients experience life threatening or fatal non-haematopoietic toxicity, and furthermore, it appears that the procedures are not sufficiently tumoricidal to ensure ablation of a haematological malignancy and neither are the regimens sufficiently immunosuppressive to ensure marrow graft acceptance.

It is also known to use chemotherapy treatment without radiotherapy generally using a combination of drugs with non-additive toxicities except on bone marrow. Common combination regimens usually include non-cross resistant

agents with different spectra of antitumour activity, such as nitrosoureas (BCNU), epipodophylotoxins (VP-16) and alkylating agents (thiotepa, cyclophosphamide or melphalan). Melphalan is a drug which has been used alone for attempts at bone marrow ablation. However, for multiple myeloma the success rate is very low for achieving complete clearance of the myeloma and there is significant morbidity and mortality.

Accordingly, it would be desirable to provide a regimen which involves less risk to the patient of life-threatening or fatal conditions and to develop a regimen which would have a high probability of achieving successful cure and not merely a remission.

The present invention is based on the concept of using a radiopharmaceutical comprising a polyvalent particle-emitting radionuclide and a chelating agent which has strong localisation on bone, the irradiation being less than the level which would be fatal in the absence of further treatment steps; administering a cytotoxic compound which affects bone marrow but at a dosage less than that would be fatal in the absence of other treatment; and after allowing for the effects of the radionuclide and cytotoxic compound to dissipate, effecting a bone marrow transplantation.

In one aspect, the invention manifests itself in a method of treatment of an animal such as a human being, and in another aspect, manifests itself in a treatment kit for the procedure.

Preferably, the radiation emits principally beta radiation which is short range but highly effective for cell ablation.

One convenient beta-emitting radionuclide is samarium-153 but there are many other possible radionuclides such as strontium-89, yttrium-90, ruthenium-103, indium-115, cerium-144, gadolinium-159, holmium-166, ytterbium-175, lutecium-177, and rhenium-186.

The selection of chelating agent referably involves a

selection of one which can be labelled with strong attachment by the radionuclide, and the resulting complex should be highly specific to bone. For example, polyaminepolyalkylphosphonic acids and derivatives including physiological salts thereof can be selected with advantage. An important example of such a compound is ethylenediaminetetramethylene phosphonate (EDTMP) which is a known chelating agent and is readily labelled with samarium-153 and has been found to localise on the surface of cortical and trabecular bone. Other chelating agents in this class are:

diethylenetriaminepentamethylenephosphonic acid (DTPMP),

hydroxyethylethylenediaminetrimethylenephosphonic acid (HEEDTMP),

nitrilotrimethylenephosphonic acid (NTMP),

tris(2-aminoethyl)aminehexamethylenephosphonic acid (TTHMP),

HEDP, or

physiologically acceptable salts of any one of these compounds.

The choice of cytotoxic compound includes melphalan (described in U.S. patent Nos. 3,032,584 and 3,032,585) and related compounds including equivalent chemotherapeutic agent with a predominantly myelotoxic action.

A preferred embodiment of the invention comprises the use of EDTMP labelled with samarium-153 administered at a high but sub-lethal level. However, it is thought that although samarium-153 labelled EDTMP is myelosuppressive, complete marrow ablation is not achieved even with very high dosage levels. Attempts to produce complete marrow ablation in dogs and rabbits have been reported as not successful, and further studies by the present inventor indicate that in rats samarium-153 EDTMP alone is unlikely to completely ablate red marrow.

Another embodiment consists in the use of rhenium-186

labelled HEDP.

Preferably, the treatment comprises delaying administration of the cytotoxic drug for several days to allow substantial radioactive decay of the radiopharmaceutical.

For illustration purposes only, reference will be now made to the accompanying drawings which illustrate trials in rats.

Referring first to Figure 1, the graph illustrates platelet concentration in the blood with time following a lethal total body irradiation in rats. The irradiation caused marrow ablation and the non-irradiated control example of rats (7 in number) maintained a substantially constant platelet concentration in blood. The irradiated sample of 5 rats showed a decline in platelet concentration in accordance with the normal decline of platelet concentation in the absence of fresh platelet generation by marrow. This model established the validity of monitoring platelet concentration as an indicator of bone marrow ablation.

Figure 2 demonstrates the viability of marrow transplantation after total body irradiation, i.e. marrow ablation. The control sample with no marrow transplantation showed all rats died within about 10 days but a very high survival rate was achieved with those that received marrow transplantation.

Figure 3 is a graph of platelet concentration with time. A lethal total body irradiation is given to the sample and marrow transplantation effected. By day 10, platelet concentration had dropped to a potentially fatal level. However, the increase in platelet concentration demonstrated that the transplantation had been effective and bone marrow cell reproduction had occurred to rescue the animals and a normal platelet concentration was achieved by day 15.

Figure 4 demonstrates the use of samarium-153 EDTMP at a rate of 3.5 GBq instead of total body irradiation.

Samarium-153 EDTMP was prepared according to published methods (Turner et al 1989 Eur.J.Nucl.Med.15: 784-795). Briefly, Samarium-153 was prepared by neutron irradiation of Sm_2O_3 (enriched to 98% Samarium-152) in the HIFAR Research Reactor, Australian Nuclear Science and Technology Organisation, using a thermal flux of 5 x $10^{13} ncm^{-2}s^{-1}$. Samarium-153 was supplied as a sterile solution of 153 Sm Cl_3 in physiological saline and was added to a lyophyllized EDTMP kit immediately prior to use. Again the control sample had a steady platelet concentration but the irradiated sample showed that although the platelet concentration had dropped close to the level at which the animal's life is threatened, spontaneous recovery occurred and this is thought to be due to the fact that the samarium-153 EDTMP may not cause complete marrow ablation.

Referring now to Figure 5, the effect on rats of melphalan at varying dose rates is indicated. It is only when dosages of around 9 mg/kg are given that survival is threatened, but then a precipitous result is observed. On this basis, in rats, a dose of about 9.5 mg/kg would be close to the fatal dose for most individuals.

Figure 6 demonstrates survival rate after chemo- and/or radiotherapy treatment comprising 9.5 mg/kg Melphalan and samarium-153 EDTMP administered at 555mBq.

A control with melphalan alone indicated 100% survival. A sample comprising the samarium and melphalan but without marrow transplantation produced a low survival rate of about 20%. The third line demonstrates a sample of 13 individuals treated with samarium-153, melphalan, and given a marrow transplant at day 3, and again a survival rate of about 20% only was achieved. This result indicates that the transplantation was not successful due to the half-life of the radionuclide, and marrow transplantation needs to be delayed until the effects of the internal endoradiotherapy have diminished.

Figure 7 indicates the result of delaying marrow transplant until six days after the commencement of the

procedure. In this case, the procedure commenced with samarium endoradiotherapy, and five days later the cytotoxic compound melphalan was administered. On day six, marrow transplantation occurred, and in the control sample which did not receive the transplantation, the survival rate was approximately 20% whereas for those individuals receiving the transplant the survival rate exceeded 90%.

CLAIMS

1. A pharmaceutical comprising a supply of bone-localising chelating agent, a supply of polyvalent particle-emitting radionuclide for labelling the chelating agent, and a cytotoxic drug, the radionuclide and cytotoxic drug being in a dosage which when administered in combination will cause bone marrow ablation in animals, each dosage being close to but less than a level which will cause complete bone marrow ablation.

2. A pharmaceutical as claimed in claim 1, wherein the radionuclide is selected from the group consisting of samarium-153, strontium-89, yttrium-90, ruthenium-103, indium-115, cerium-144, gadolinium-159, holmium-166, ytterbium-175, lutecium-177, and rhenium-186.

3. A pharmaceutical as claimed in claim 1 or claim 2 wherein the radiopharmaceutical is selected from EDTMP, DTPMP, HEEDTMP, NTMP, TTHMP, HEDP and physiologically acceptable salts thereof.

4. A pharmaceutical as claimed in any one claims 1 to 3 and wherein the cytotoxic drug is melphalan or a derivative or analogue thereof, or a chemotherapeutic agent with a predominantly myelotoxic action and substantially equivalent to melphalan.

5. A method of treating haematological malignancy in an animal being comprising administering a bone-localising radiopharmaceutical labelled with a polyvalent particle-emitting radionuclide to affect substantially bone marrow of the animal, administering a cytotoxic pharmaceutical in a dose sufficient to affect substantially bone marrow of the animal, the combined doses of the radio pharmaceutical and the cytotoxic pharmaceutical being chosen to cause bone marrow ablation, after a delay sufficient to allow substantial decay of the radiopharmaceutical, effecting a bone marrow transplantation.

6. A method as in claim 5 wherein the radiopharmaceutical is labelled with a radionuclide selected from the group consisting of samarium-153, strontium-89, yttrium-90, ruthenium-103, indium-115, cerium-144, gadolinium-159, holmium-166, ytterbium-175, lutecium-177, and rhenium-186.

7. A method as in claim 5 or claim 6, wherein the radiopharmaceutical is selected from EDTMP, DTPMP, HEEDTMP, NTMP, TTHMP, HEDP and physiologically acceptable salts thereof.

8. A method as in any one of the claims 5 to 7 wherein the cytotoxic drug is melphalan or a derivative or analogue thereof or a chemotherapeutic agent with a predominantly myelotoxic action and substantially equivalent to melphalan.

9. A method as in any one of claims 5 to 8 wherein the cytotoxic drug is administered a few days after the radiopharmaceutical, and the bone marrow transplantation is delayed of the order of a day after administering the cytotoxic drug.

LETHAL TOTAL BODY IRRADIATION IN WAG RATS

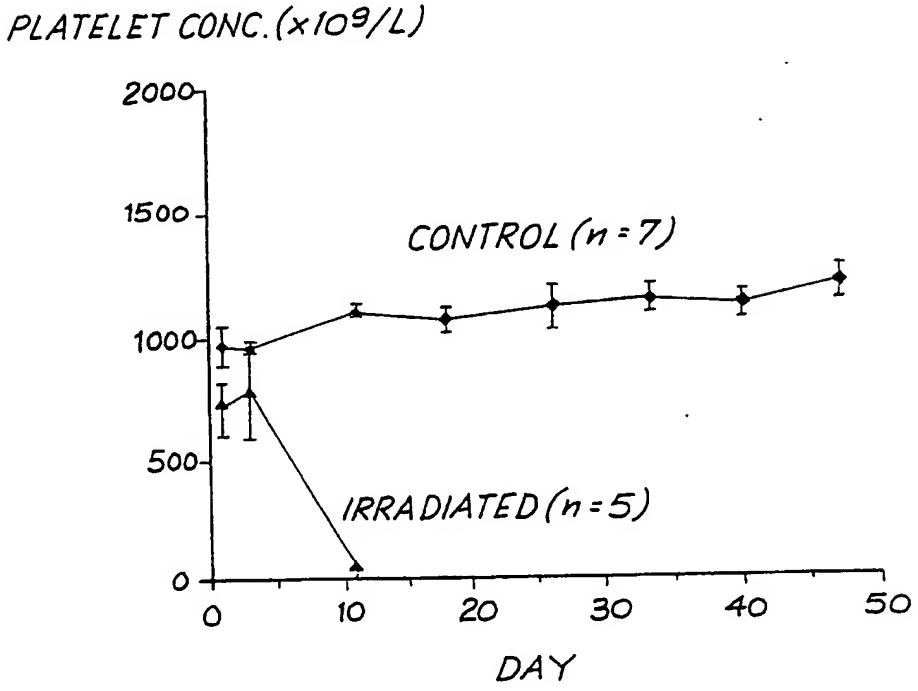

FIG. 1

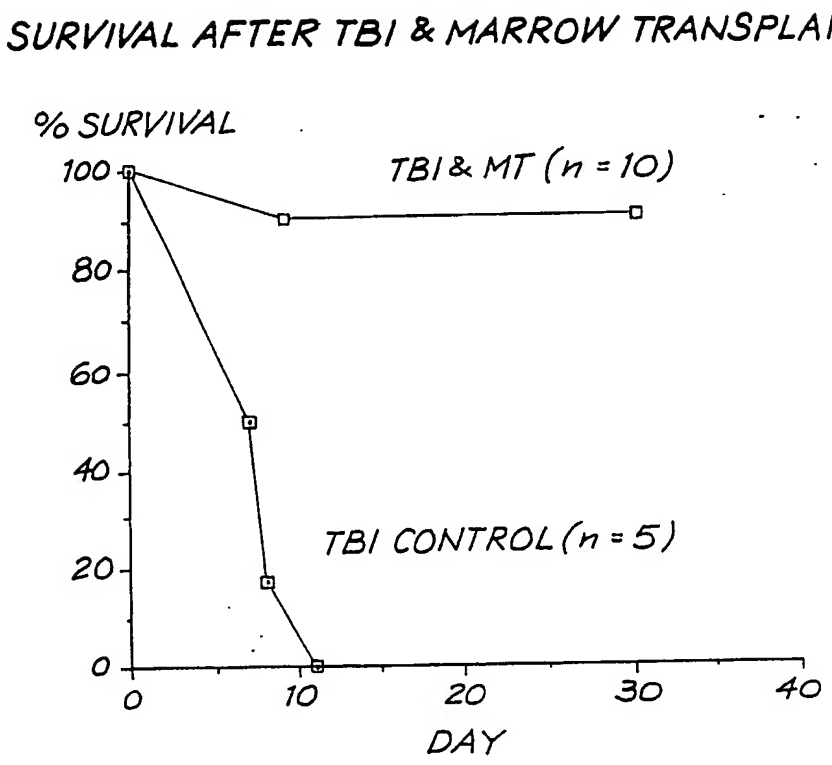
SURVIVAL AFTER TBI & MARROW TRANSPLANTATION
% SURVIVAL
100
80
60
40
20
0
TBI & MT (n = 10)
TBI CONTROL (n = 5)
0
10
20
30
40
DAY

FIG. 2

MARROW TRANSPLANTATION AFTER LETHAL TBI

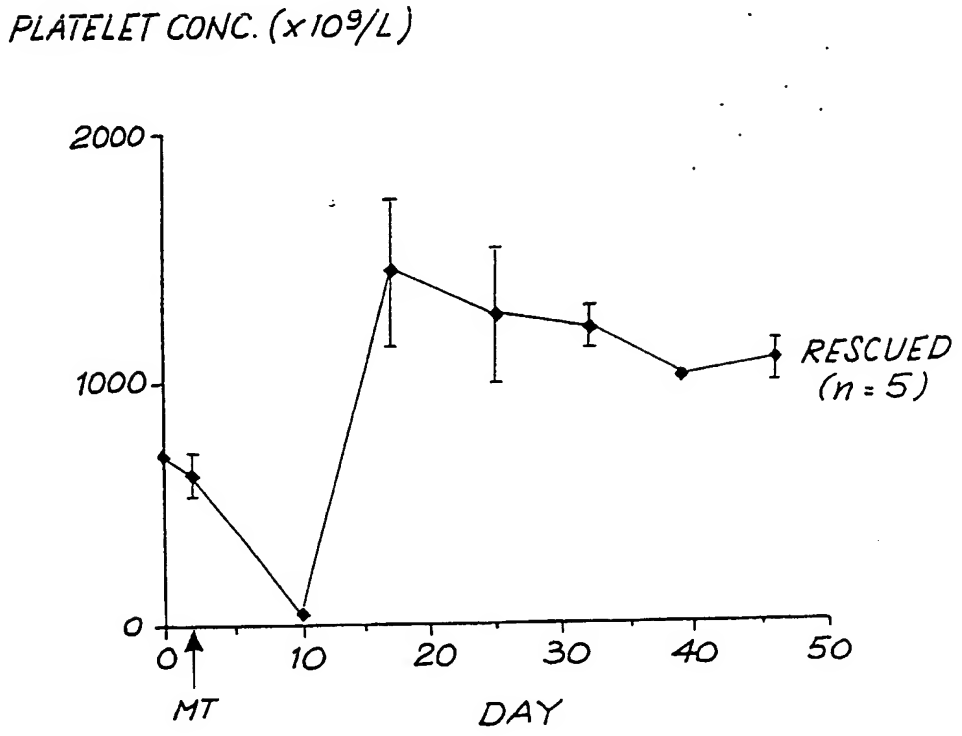

FIG. 3

IRRADIATION BY SAMARIUM-153-EDTMP : 3.5 GBq IV.

PLATELET CONC. ($\times 10^9$/L)

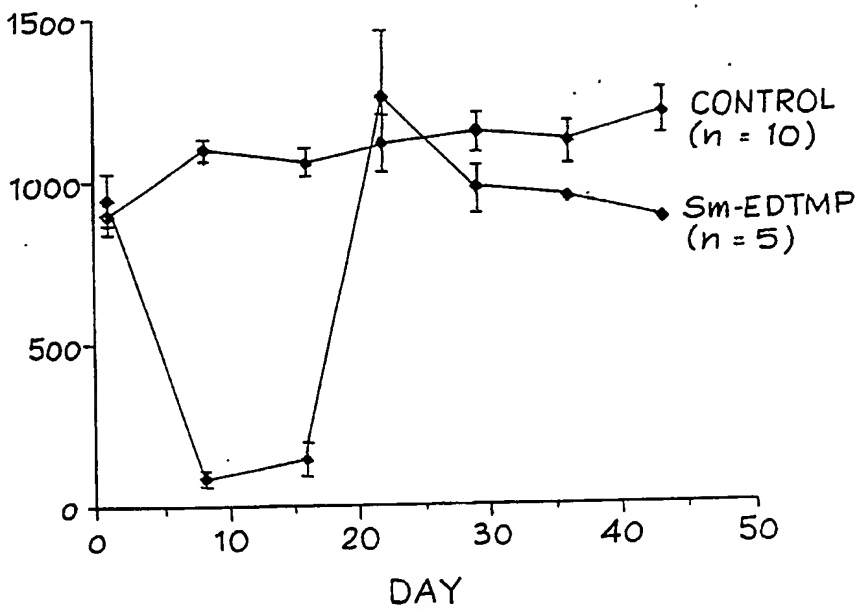

FIG. 4

SURVIVAL AFTER 0.5 -10.0 mg/kg MELPHALAN IP.

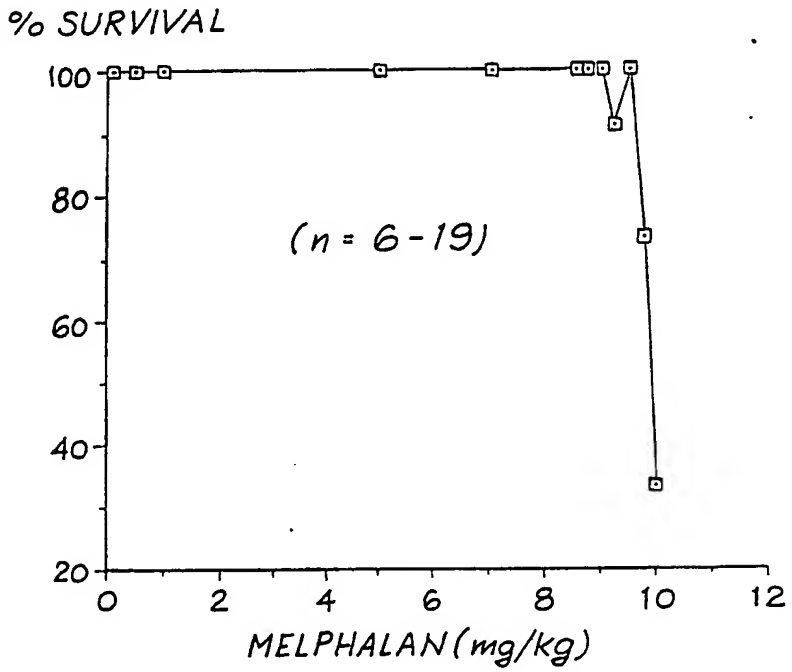

FIG. 5

SURVIVAL AFTER CHEMORADIOTHERAPY:
9.5mg/kg MELPHALAN and 555 mBq/kg 153Sm-EDTMP

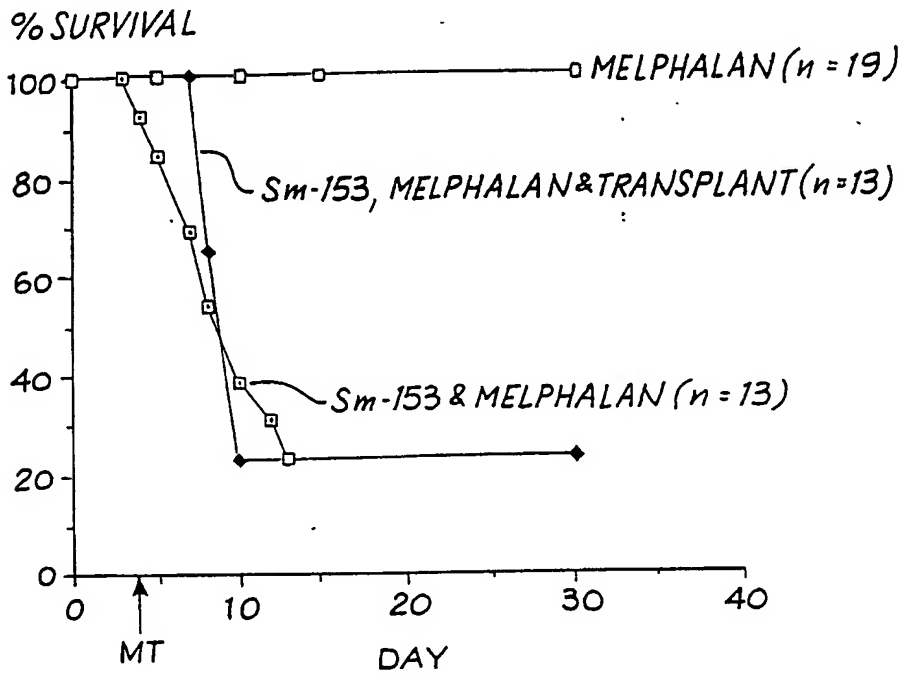

FIG. 6

SURVIVAL AFTER SEQUENTIAL CHEMORADIOTHERAPY:

555 mBq/kg 153 Sm EDTMP DAY 0
9.5 mg/kg MELPHALAN " 5
MARROW TRANSPLANTATION " 6

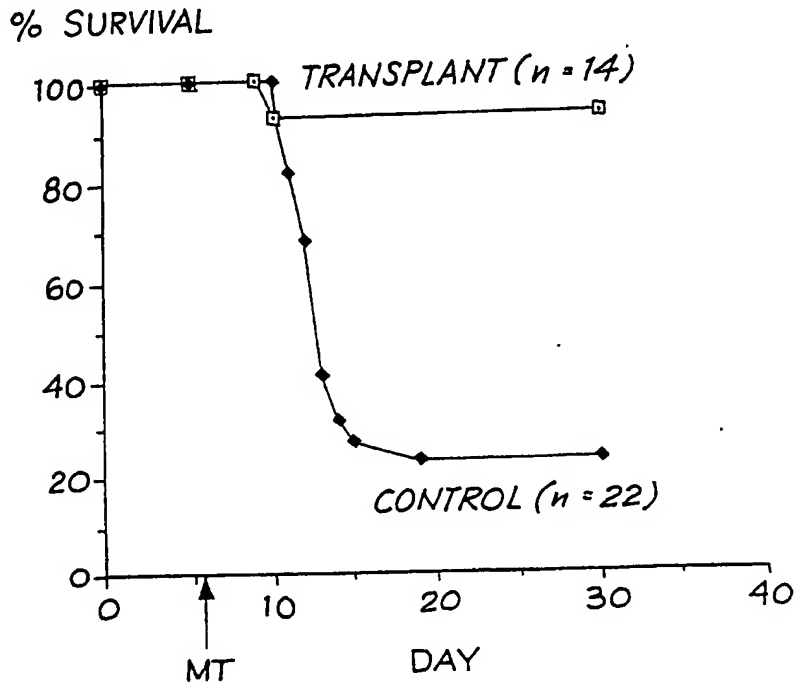

FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 91/00155

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] A61K 43/00, A61K 31/195

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC	A61K 43/00, A61K 31/195

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

AU : IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X	US,A, 4853209 (KAPLAN et al.) 1 August 1989 (01.08.89) See entire document, claims 1,9,11,12,17,18.	(1-9)
X	US,A, 4882142 (SIMON et al.) 21 November 1989 (21.11.89) See entire document, claims 1,5,7,8,13,14.	(1-9)
X,P	US,A, 4976950 (SIMON et al.) 11 December 1990 (11.12.90) See entire document, claims 1,3,5,6,7,10.	(1-9)
A	EP,A, 164843 (THE DOW CHEMICAL COMPANY) 18 December 1985 (18.12.85) See claim 1.	(1-3)
A,P	EP,A, 375376 (THE DOW CHEMICAL COMPANY) 27 June 1990 (27.06.90) See claim 1.	(1-3)

(continued)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2 August 1991 (02.08.91)	13 August 91
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	TAMARA NIZNIK

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	US,A, 3965254 (TOFE et al.) 22 June 1976 (22.06.76) See entire document, column 9 lines 36-68.	(1-4)
A	'Goodman and Gilman's The Pharmacological Basis of Therapeutics' eds A.G. Gilman et al., seventh edition, published 1985, by Macmillan Publishing Company (New York) see page 1243.	(1-9)

V. [ ] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.[ ] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2.[ ] Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.[ ] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1.[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00155

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Patent Document Cited in Search Report	Patent Family Members		
US 4853209	AU 80453/87 JP 63287729 ZA 8708169	DK 5706/87 NZ 222304	EP 291605 PT 86020
US 4882142 US 4976950	AU 45440/89 DK 5827/89 EP 374501 JP 2237936		
EP 164843	AU 41229/85 JP 61022029 US 4898724	CA 1243603 NZ 211808	IL 74902 ZA 8502799
EP 375376	AU 47009/89 CN 1046739 HU 54897	AU 48282/90 DK 1959/90 NO 903632	BR 8907255 EP 408701 WO 9006776
US 3965254	AU 69210/74 DE 2424453 NL 7406952	BE 815397 FR 2230374 PH 12898	CA 1028246 GB 1453667 ZA 7403159

END OF ANNEX

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00155

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US 4853209	AU 80453/87, DK 5706/87, EP 291605, JP 63287729, NZ 222304, PT 86020, ZA 8708169
US 4882142, US 4976950	AU 45440/89, DK 5827/89, EP 374501, JP 2237936
EP 164843	AU 41229/85, CA 1243603, IL 74902, JP 61022029, NZ 211808, ZA 8502799, US 4898724
EP 375376	AU 47009/89, AU 48282/90, BR 8907255, CN 1046739, DK 1959/90, EP 408701, HU 54897, NO 903632, WO 9006776
US 3965254	AU 69210/74, BE 815397, CA 1028246, DE 2424453, FR 2230374, GB 1453667, NL 7406952, PH 12898, ZA 7403159

END OF ANNEX